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NUTRITION REVIEWS

VOL. 18 MARCH 1960 No. 3

THE OPERATION OF A METABOLIC WARD

Occasionally it is valuable to review the origin and evolution of accepted practices in clinical investigation, just as it is well to take an occasional critical look at anything which has been accepted as common practice.

The development of balance studies in man under complete control in a metabolic ward is a feature of contemporary clinical investigation. The elaborate facilities we use today are in sharp contrast to those used in 1913 by Graham Lusk and Eugene DuBois or to the resources described by Walter Bauer and Joseph Aub (*J. Am. Dietet. Assn.* **3,** *106 (1927)*). Yet these early research wards, designed to permit rigorously accurate collections and observations, were forerunners of the modern research ward, which is in effect a clinical laboratory. The development of research techniques has been related by E. C. Reifenstein, F. Albright and S. L. Wells (*J. Clin. Endocrinol.* **5,** *367 (1945)*).

Anyone with experience in the foibles of a well-run metabolic ward, the uniqueness of the human individual and the difficulty of using many subjects, is acutely aware of the impossibility of obtaining the necessary precision of diet, sample collection, observation and recording of signs and symptoms in even the best regulated general ward or private hospital room. The frustrations, complexities and confusion occurring at various times during the development of our metabolic ward have given us a background against which to review our current practices. Despite the most rigid and scrupulous attention to detail, errors and missed collections continue to pose vexing problems.

Periodically we evaluate our methods in the light of others' experience. Recently we corresponded with the directors of metabolic research in 18 different medical centers, asking them to share with us their methods and experience. From 12 institutions we obtained detailed and comprehensive analyses. It was not surprising that all stressed a belief that an intelligently cooperative patient or subject was the primary requirement. It was agreed that this was more important than the special techniques of collecting and measuring specimens or using markers to identify time relations in dietary or experimental programs. Most agreed that metabolic studies conducted on service wards were fraught with a 50 per cent error.

Our own metabolic unit is constructed as follows:

Physical Facilities

The metabolic unit has three large rooms, each capable of housing three patients in comfort. Across the hall are the nurses' office, an examining room, a utility room, a clinical laboratory and storage space. Nearby is a metabolic kitchen equipped to provide any type of diet or formula. A separate pair of bathrooms and toilets are equipped for collection of urine and feces. Refrigerators and freezers occupy the foyer outside these toilets. For diversion, a recreation room has tables and chairs for dining, a television set, games, crafts, books and magazines. Down the hall are the nutrition laboratories and the research laboratories of several clinical investigators.

Staff

Because the facilities of the metabolic unit are available to all members of our clinical staff, the investigators change from time to time. A committee of four persons acts upon proposals for projects and assists

informally in the supervision and conduct of each study. Most studies are conducted by one or more members of this committee, whose main interests are nutrition, metabolism, endocrinology, cardiac disease and gastroenterology.

Nursing care of high caliber is provided by a staff of registered and practical nurses. All have been indoctrinated in the principles of investigative methods and the necessity for attention to minute details. They keep accurate records of daily activity, complaints, symptoms and behavior. Great emphasis has been placed on absolute honesty since errors occur despite scrupulous care. Such errors must be known.

Dietetic services provide more than the formulation and serving of prescribed diets, although these are important. Dietitians assist in planning all studies and conduct many of their own. They are skilled in performing many biochemical tests and are most helpful in evaluating and interpreting data.

The laboratories are staffed by technicians, graduate students and assistants, who learn to perform all manner of tests, some simple and routine, some elaborate and complex.

Patient Material

The people who serve as subjects on our metabolic ward are of three types. (1) Patients in the hospital who have a disease or condition of particular interest. (2) Healthy volunteers (usually medical or dietetic students) who may be studied as outpatients. (3) Healthy volunteers from a state penal institution who have been selected because of willingness, stability of personality and good health.

Policies of Operation

Often colleagues whose interests lie in other regions of medicine speak with disparaging words of a "metabolic study" as though it were slightly degrading. To them it may mean an attempt to reduce a fat patient or a routine testing of a new therapeutic agent. While either of these might be the subject of an investigation, it is essential that all persons participating in a metabolic study should understand the necessity for a program planned to the smallest detail. By studying the work of others and planning methods of his own, the erstwhile investigator either gains in enthusiasm or quits in despair.

A protocol must be prepared and reviewed informally by the committee. This policy allows us to dissuade the casual investigator as well as help the enthusiastic young clinician strive for excellence through sound planning and precise execution, allowing him to mature in an environment of friendly but critical assistance. By the same token, nurses learn details, methods and principles not taught in their usual curriculum, and dietetic students practice their skills while performing investigations of their own, usually in conjunction with a larger project.

The details of routine operation are simple and strict. Each patient is under the control and supervision of a physician who records a detailed history and physical examination, examines the patient daily, makes progress notes and writes orders for all medications and changes of routine. He also performs all venipunctures and special procedures.

The nurses, in addition to their routine duties of patient care, must observe the patients continuously. This reduces the risk of loss of specimens, eating of unauthorized food or any other disruption of the experimental program. They also collect, measure, record, label and preserve all specimens which are to be saved, and distribute aliquots of them to the appropriate laboratories. The dietitians furnish exactly measured food on attractive trays, or formulas disguised as well as possible. They participate in all phases of the study from planning, to chemical determinations, evaluation of data, and reporting of results.

All records are filed permanently in the metabolic ward. None is sent to the hospital record room. This policy requires some duplication of records when ill patients are

studied, but it has avoided the irksome irritation of the lost chart.

The last and most important aspect of operation deals with the subject or patient. Despite relentless effort and great expense, no successful study can be performed without enthusiastic and understanding cooperation from him. Careful selection and complete explanation and detailed instruction are necessary to insure any measure of success. The volunteer must be a free agent, but frequent encouragement and friendly reminders when mistakes occur serve to impress upon him the necessity for compliance and honesty in every action.

When confusion arises it almost invariably stems from lack of understanding. To reduce this the staff holds informal weekly conferences to discuss everything concerning the investigation. All routines, diets, medications or tests, and any changes are recorded in the chart, and each member of the team is notified. These measures, together with frequent discussions, help maintain smooth operation.

The cost of operating a metabolic unit such as ours is staggering. The average occupancy of between five and six patients daily throughout the year taxes the facilities of our staff and laboratories to the utmost. To calculate the daily cost per patient one must subdivide expenses into several categories. If one considers only room and board, the figure is approximately $11.00 daily. But if one considers salaries, cost of equipment and operating laboratories and the many other items of expense, our cost is more than $50.00 per bed per day.

Any patient assigned to our metabolic ward retains the status he had in the general hospital, as a private, semi-private or indigent patient. In the case of volunteers from a prison, the hospital administrators have generously provided routine facilities.

The financial support for the ward has been derived in large measure from grants in aid from the National Institutes of Health, The Nutrition Foundation, The National Vitamin Foundation and The American Cancer Society, supplemented by smaller grants from pharmaceutical companies and other sources.

Is such expense extravagant and unwarranted? Sometimes we wonder. Nevertheless, a metabolic ward is the only facility that can control the subject, provide a stable environment, a precise diet and accurate collection of specimens. Although the cost is great, the results in terms of reliable information justify the expense. Even diseases of the cardiovascular or gastrointestinal system must be studied by such methods if they are to be fully understood, and perhaps this applies to other diseases as well. More important, however, is the training of a corps of physicians, nurses, dietitians and technicians in rigorous methods which may yield important answers in the future.

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VASCULAR DISEASE AND THE GROWTH, REPAIR, REGENERATION AND DEGENERATION OF VASCULAR ELASTIC MEMBRANES

The vascular lesions resulting from remodelling or repair of the arterial walls and factors controlling local fibrin deposits are involved in degenerative arterial diseases.

Several interesting aspects of the genesis of degenerative arterial diseases seem to arise from the recent studies in this field by T. Gillman and co-workers in South Africa.

These workers have repeatedly expressed opinions concerning the genesis of arterial diseases which, while incorporating the ideas expressed by several other workers, have also

added some new ideas and facts. They maintain that occlusive diseases are probably the result of two distinct but usually concurrent processes, *i.e.*, injury to the arterial walls themselves culminating in vascular sclerosis, and the intra-arterial thrombotic processes, probably occurring repeatedly, particularly at sites of previous vascular injuries and the scars resulting therefrom (*Lancet* **II,** *1117 (1957);* **II,** *901 (1958)*).

Pursuing the problems along these two lines, Gillman and co-workers have been analyzing, first, the basic structure of vascular elastic membranes, their mode of growth and reactions to injury, and second, the intravascular factors which might play important roles in promoting or retarding mural thrombotic or fibrin deposits over sites of previous injuries and the effects on these of fat intake and sex hormones.

Their studies of the structure and postnatal development of the vascular elastic membranes, their reactions in human and experimentally induced lesions indicate that arterial elastic membranes are not homogenous in structure, *i.e.*, they seem not to be comprised solely of "pure" elastic tissue, in contrast to what is the apparent case for the ligamentum nuchae. They suggest that the vascular elastic membranes are comprised of "collagen-like" hyaline "cores" enclosed by an elastic-like component, this whole complex, in turn, being sleeved by an irregular coat of mucopolysaccharide. This opinion is based upon their extensive morphological and histochemical studies of the vascular elastic membranes in man and rats at different ages and in various pathological states (*A.M.A. Arch. Path.* **59,** *733 (1955);* **67,** *624 (1959); Connective Tissue, p. 128. Blackwell, Oxford (1957); J. Mt. Sinai Hosp.* **24,** *857 (1957)*).

Their recent analysis led these investigators to suggest that, in man, the vascular elastic membranes are remodelled during growth, an opinion to which they were initially led from studies of *Lathyrus*-intoxicated rats (see below). Such remodelling may depend upon a continuous interchange of the molecular components of the various parts of the vascular elastic membranes, a process which may involve in some way the irregular perimembranous sleeves of mucopolysaccharide. They record that alterations in certain tinctorial reactions of elastic-like components of vascular elastic membranes, which occur spontaneously even in infants (perhaps as part of normal growth and remodelling) are almost invariably associated with increases in the mucopolysaccharide surrounding the areas of change (*A.M.A. Arch. Path.* **67,** *624 (1959)*).

These workers also noted a progressive series of associated changes in extracellular peri-membranous fat, in intimal cellularity, amount of mucopolysaccharide and in the staining of the affected vascular elastic membranes. These changes seem to culminate in marked intimal thickening and patchy sclerosis and so-called "reduplication" of the related vascular elastic membranes. They consider that "reduplicated" vascular elastic membranes are not comprised of "true" tissue but of what they call "pseudo-elastic" tissue (*A.M.A. Arch. Path.* **59,** *733 (1955);* **67,** *624 (1959)*).

The vascular elastic membranes seem to be replaced by regeneration during normal growth and aging of arteries. Such regeneration is distinguishable from repair by certain definable criteria. "Reduplication," or rather appearance of "pseudo-elastic" membranes seems to indicate some derangement in normal regenerative processes with resulting architectural disorder, *i.e.*, repair.

These investigators suggest that terminal sclerotic lesions in the arteries of young adults may represent the late end result of earlier deranged remodelling, which probably first occurs during the rapid growth of arteries at adolescence or even during infancy. The sclerosis of arteries in aged individuals, on the other hand, may reflect the gradual deterioration of the individual's capacity to regenerate the wear and tear changes normally occurring in all arteries. Repair processes then ensue with unavoid-

able and progressive scarring as the outcome (*Lancet* **II,** *1117 (1957);* **II,** *901 (1958)*).

This approach may be of some value in studying, for example, the arterial changes in diabetics. For it is now recognized that diabetes must be present for some years before arterial and capillary pathology become manifest. Could the overall disturbances in carbohydrate metabolism in long-standing diabetes find some expression also in altered peri-membranous and other connective tissue carbohydrate turnover (such as is in the mucopolysaccharide sleeves), thus leading to the pathological changes characteristic of advanced diabetes? This possibility is supported by H. Grossfield's recent analysis of the role of carbohydrate metabolism in maintaining the structural proteins in arteries (*Nature* **184,** *38 (1959)*).

Gillman and associates suggest that the repair of vascular elastic membranes, injured acutely by single short episodes of calciferol intoxication in rats, closely simulates that observed in healing cutaneous injuries. Their data indicate that such arterial injuries are, however, repaired only slowly and that even single episodes of acute arterial injury, if severe enough, may culminate only months later in rats (and perhaps only years later in man) in vascular scarring, *i.e.*, sclerosis. Thus they suggest that some forms of arteriosclerosis in man, especially in certain arteries (*e.g.*, coronaries) may simply be the late end result of single episodes of acute arterial injuries incurred traumatically or metabolically.

Furthermore, their studies of *Lathyrus*-intoxicated rats led them to suggest that the arteries of the rat are most susceptible to the toxic action of nitriles at a time when these arteries are growing most rapidly in length and diameter (and therefore being remodelled), particularly during the first few weeks post-natally or the last week of intra-uterine life (*J. Embryol. Exp. Morph.* **6,** *270 (1958); Schweiz. Z. Path. Bakt.* **22,** *62 (1959)*). The way in which such vascular growth, which must be occurring at these times, is achieved has still not been divulged, but it may perhaps be accompanied by a process comparable to that seen in long bones, *i.e.*, by endarterial (endosteal) lysis, closely coupled with intramedial or adventitial (periosteal) neogenesis of arterial membranes (bone).

In the view of these investigators, the aortic ruptures in *Lathyrus* intoxication may be due, not so much to increased lysis, but rather to depressed neogenesis of vascular elastic membranes, an opinion supported by recent observations that collagen synthesis is depressed by nitrile intoxication (*Schweiz. Z. Path. Bakt., loc. cit.*). Such alterations in the synthesis of "collagen-like" components of vascular elastic membranes may involve some block either in the synthesis of the pro-collagen or, alternatively, in the linking of the submicroscopic units of collagen fibrils.

Their work thus provides a further stimulant for the re-examination of the architecture of vascular elastic membranes, especially by the use of electron microscopy. Analysis of the mechanism of regeneration of these vascular elastic membranes, and especially of their remodelling as the arteries grow during adolescence, seems particularly worthy of study with radioactive isotopes. A similar approach to the study of regeneration and repair of vascular elastic membranes in various diseases, *e.g.*, in long-standing diabetes, may also provide data of great value for our understanding of degenerative arterial diseases.

These investigators also suggest that the earliest injuries to human arteries may be initiated most readily when the vessels are growing and being rapidly remodelled, especially during adolescence. They propose that the deterioration of such early initial arterial injuries into frank vascular scars probably takes years. R. L. Holman *et al.* (*Am. J. Path.* **34,** *209 (1958)*) have recently shown that endarterial fatty streaks appear most frequently during adolescence, while intimal scars develop only ten to 15 years later.

Such deterioration of early injuries to

scars and atheromatous plaques may be facilitated by the sexually determined differences in fat metabolism, plasma fibrin and fibrinolytic activity and by the sexually different responses of plasma fibrinolysins to fat intake, such as were found by Gillman and co-workers in rats (*Endocrinology* **62,** *92 (1958); Clin. Sci.* **17,** *393 (1958)*).

They also found plasma fibrinolytic activity in man to be altered considerably during pregnancy and the puerperium (*Lancet* **II,** *70 (1959)*), as well as in liver disease (*Brit. Med. J.* **II,** *43 (1957)*). They suggest this may be due to fluctuations in endocrine balance. Moreover, their studies with Africans with liver disease, as well as those of H. C. Kwaan and A. J. S. McFadzean with Chinese with hepatic cirrhosis (*Lancet* **I,** *136 (1956)*), indicate that markedly elevated plasma fibrinolytic activity is frequent in patients with chronic hepatic damage and in pellagrins, again perhaps attributable to the altered endocrine balance in cirrhotics and pellagra. The latter possibility seems likely in view of Kwaan's and McFadzean's demonstration (*Lancet* **I,** *136 (1956)*) that ACTH therapy in cirrhotics results in a decrease in plasma fibrinolytic activity which is so profound and rapid as to be followed, in some cases, by portal thrombosis.

Gillman and co-workers suggest that, in the presence of vascular injuries of developmental origin, the already low plasma fibrinolytic activity (apparently hormonally determined in males) may perhaps be further depressed by regular high fat intakes, thus promoting (particularly in males) endarterial fibrin silting such as that described by J. B. Duguid (*Brit. Med. Bull.* **II,** *36 (1955)*), especially at sites of previous arterial injuries (*Lancet* **II,** *1117 (1957)*; **II,** *901 (1958)*). Malnutrition may decrease endarterial silting not only by its effect on blood lipids but also by associated endocrine changes (*Ciba Foundation Colloquia on Aging* **3,** *104, 188 (1957); Lancet* **II,** *696 (1957)*).

These workers are thus of the opinion that the pathological changes within arteries will predispose to actual occlusive episodes only under appropriate metabolic conditions. They therefore favor a multiple factor etiology of the occlusive episodes in association with previously established coronary pathology of developmental origin. They consider that their own observations may provide an explanation for the different frequencies of coronary occlusive episodes in different races, despite the almost equal incidence of pathological changes in coronary and other arteries in different populations recently described by Holman and co-workers (*Am. J. Path.* **34,** *209 (1958)*).

The possibility that hormonally induced changes may play an important role in maintaining the integrity of arteries is supported by their observations that human uterine arteries seem to undergo profound remodelling during pregnancy and that the higher incidence of arterial diseases found by them in old breeding female rats as compared with non-breeding females or with males of the same ages (*Lancet* **II,** *901 (1958); Nature* **183,** *1139 (1959)*) may be attributable in part to the greater vascular remodelling occurring during pregnancy. They indicate the need for determining the relative incidence of degenerative arterial diseases in human virgins and mothers at different ages.

In summary: These investigators believe that there is probably no single cause of degenerative arterial diseases or of terminal thrombosis. The final common path probably involves two independent but usually concurrent processes: (1) the vascular lesions resulting from deranged growth, remodelling, regeneration or repair of acute injuries to arterial walls themselves, and (2) the factors in the circulating blood (influenced by diet and hormone balance) which promote or suppress localized intimal fibrin deposits, apparently most often formed over rigid intramural vascular scars (*Excerpta Medica, Sect. XX,* **2,** *367 (1959)*).

FASTING AND ELECTROLYTES (ANOREXIA NERVOSA)

Patients with anorexia nervosa show a low serum potassium level (especially with excessive weight loss) accompanied by reduced levels of sodium and chloride and elevated levels of carbon dioxide. High intakes of potassium overcome these abnormalities.

Anorexia nervosa is a relatively rare disease occurring predominantly in women between the ages of 15 and 40 years (*Nutrition Reviews* **11,** *271* (*1953*)). The individuals afflicted with this disease frequently starve themselves to death. There is at present no satisfactory etiological explanation for the condition. Psychiatric treatment has been the only therapy which has shown any success, but complete recovery has occurred in only a small percentage of the cases.

J. R. Elkinton and E. J. Huth (*Metabolism* **8,** *376* (*1959*)) at the University of Pennsylvania recently reported on the disturbances in electrolyte metabolism in patients with anorexia nervosa. They stated that since 1938 only 20 cases of anorexia nervosa had been seen at the University Hospital. For these patients, Elkinton and Huth plotted the total carbon dioxide content of the serum against the per cent of body weight loss. They found that the values were within normal limits except when the weight loss exceeded 25 per cent, in which case the level of carbon dioxide was higher than normal. When similar graphs were made for the serum levels of chloride, sodium and potassium, these electrolytes were also within normal limits except when the body weight loss exceeded 25 per cent. In the latter situation the levels of the electrolytes were below normal.

To establish the nature and the mechanisms responsible for the alterations in electrolyte concentrations, Elkinton and Huth kept two patients with anorexia nervosa in a metabolic ward. One patient was 34 years old and weighed 35.5 kg. (56 per cent of her initial weight), and the other was 43 years old and weighed 32.6 kg. (65 per cent of her initial weight). Each patient was given a standard, neutral ash diet which provided about 30 mEq (0.12 g.) of potassium per day. On this basal diet, there was a retention of 23 mEq of potassium. When the patients were given increasing amounts of potassium (as the chloride), they retained most of it even at intake levels of 160 mEq (6.2 g.).

During the period of potassium administration, the serum potassium levels increased from 2.5 mEq per liter to normal values of 3.6 to 4.4 and there was a reduction to normal of the serum carbon dioxide content. However, there was no essential change in the nitrogen balance. Potassium was retained in excess of nitrogen, with potassium to nitrogen ratios as high as 15.1 compared to the normal range of 2.4 to 3.0.

The retention of potassium was associated with the retention of chloride as shown by the increases in the chloride space, ranging from 2.5 liters to 11.9 liters in the different experimental periods. These changes were far greater than the changes in body weight (-0.68 to $+2.10$ kg.).

The above observations suggested to Elkinton and Huth that there was a higher concentration of chloride in some body fluid other than plasma. However, they pointed out that this suggestion is open to question since their subjects may have surreptitiously discarded their vomitus and thus vitiated the calculated chloride balance. Individuals with anorexia nervosa show a high tendency to vomit and are reluctant to acknowledge this fact (Elkinton and Huth, *loc. cit.*).

The hyponatremia originally present in both patients was corrected during the period of positive potassium balance, and this correction in sodium balance was achieved in the absence of an increased sodium intake.

The marked hypochloremia alkalosis and hypokalemia seen in the two anorexia nervosa patients may very well have been the

result of vomiting, since kidney and adrenal functions appeared to be normal. The normal adrenal activity was shown by a normal 24-hour urinary excretion of aldosterone and other alpha-ketolic steroids. Additional evidence for normal adrenal activity was provided by J. M. Berkman, C. A. Owen, Jr., and T. B. Magath (*Postgrad. Med.* **12,** *407 (1952)*), who studied a 36-year-old woman whose height was 156.2 cm. and whose weight was 26.3 kg. Her 24-hour urine sample contained 1.4 mg. of 17-ketosteroids and 0.42 mg. of corticosteroids. Both of these values were normal.

Hypokalemia as severe as that seen in the above patients was not observed in any of the ten patients with anorexia nervosa studied by H. Ljunggren, D. Ikkos and R. Luft (*Acta Endocrinol.* **25,** *290 (1957)*). Two of their patients had plasma potassium values of 2.9 and 3.0 mEq per liter as well as low plasma sodium levels. One had low chloride levels.

The above studies on patients with anorexia nervosa emphasize the marked differences that are likely to occur in the electrolyte balances, even when these patients are kept in a metabolic ward. Part of this probably stems from the difficulty in securing complete cooperation due to the disturbed psychological state of these patients. Their tendency to surreptitiously discard their vomitus poses a possible loophole which should be carefully checked if metabolic studies are to be carried out.

The outstanding characteristic of the electrolyte disturbances in these patients is the tendency for a low level of serum potassium to develop as they lose more than 25 per cent of their initial body weight.

SYNDROMES OF MAGNESIUM DEPLETION AND RETENTION IN MAN, PART I.

A syndrome of magnesium deficiency probably can be defined in certain patients with alcoholism or electrolyte imbalance. This consists of sudden psychoses, neuromuscular hyperirritability and electrocardiographic changes.

Although the body contains about 21 g. of magnesium (11 g. in the skeleton and 9.5 g. in the cells) only 0.5 g. is found in extracellular water, about 2 mEq per liter of plasma. Our knowledge of magnesium distribution and metabolism is much less than of many other electrolytes, no doubt because it is such an insignificant part of the extracellular fluid electrolyte total and because until recently its measurement has been tedious. Nevertheless it is the second most abundant intracellular cation, comprising about 6 to 20 mEq per liter compared to about 120 mEq per liter for potassium. Renal conservation is excellent and, as with other electrolytes, the plasma level probably bears little relation to the intracellular concentration, so that it is not surprising that syndromes attributable to changes in magnesium have been poorly correlated with its plasma concentration.

An excess of magnesium given parenterally leads to narcosis, which is completely reversed within seconds after sufficient calcium salt is given intravenously. Magnesium is important in neuromuscular conduction of skeletal and cardiac muscle; it activates numerous enzymes involved in carbohydrate metabolism and is required for the action of a variety of other intracellular enzymes. It has long been known as a component of chlorophyll, in whose structure it holds the same position that iron does in heme.

Increased serum magnesium levels have been observed in acute renal failure by W. E. C. Wacker and B. L. Vallee, using a multichannel flame spectrometer (*New Engl. J. Med.* **257,** *1254 (1957)*). They point out

the similarity of symptoms of uremia and of hypermagnesemia and suggest that the latter may account for some of the weakness and somnolence of the former. Since the electrocardiographic and clinical signs of hyperkalemia and hypermagnesemia are also similar, the authors thought that the latter might occur in acute renal failure and be responsible in part for the symptoms of "potassium intoxication." Indeed, some cases of this syndrome, with little increase in serum potassium, did prove to have hypermagnesemia. In general, however, increases of serum magnesium roughly paralleled those of potassium.

A deficiency syndrome has been experimentally studied in dogs and rats, which develop vasodilatation, cardiac arrhythmia, hyperirritability, spasticity, convulsions and death (*Nutrition Reviews* **17,** *112 (1959)*). Although some of the physiologic and biochemical actions of this element are well known, a magnesium deficiency syndrome in man has not been clearly defined and clinically recognized in the past.

The syndrome of "grass staggers" or grass tetany of cattle is associated with a sudden decrease in serum magnesium concentration and is partially preventable by fertilization of grassland soil with magnesium (*Nutrition Reviews* **12,** *214 (1954)*). Since the grass is not magnesium-deficient in the first place, the syndrome does not represent a simple deficiency. Likewise magnesium deficiency develops in calves fed too long on milk, apparently because of a progressively lesser utilization of dietary magnesium (*Ibid.* **13,** *12 (1955)*; **16,** *173 (1958)*).

Other aspects of magnesium metabolism have been reviewed in this journal (*Nutrition Reviews* **12,** *77, 181 (1954)*; **15,** *237 (1957)*). Wacker and Vallee have published a comprehensive review with 232 references (*New Engl. J. Med.* **259,** *431, 475 (1958)*).

E. B. Flink *et al.* have published an article entitled "Evidences for Clinical Magnesium Deficiency" (*Ann. Int. Med.* **47,** *956 (1957)*). They describe "a clinical syndrome characterized by muscle tremor, twitching and more bizarre movements, occasionally by convulsions and often by delirium," which they attribute to magnesium deficiency. They believe that deficiency of this element is a not uncommon clinical problem with "distinctive clinical features."

The authors studied "a large group of patients with chronic alcoholism and tremulousness and a few postoperative patients and patients with pyloric obstruction and alkalosis." They measured serum and urine magnesium levels and obtained partial balance data in some. Results are reported in 29 patients with tremor and delirium (mean serum magnesium 1.58 ± 0.3 mEq per liter), and in another group of 21 patients with tremor and muscular twitching without delirium (mean serum magnesium 1.88 ± 0.4 mEq per liter). The authors' previous measurements on "normal subjects and patients without neurologic manifestations" gave a mean value of 227 ± 0.26 mEq per liter. Four illustrative cases are reported, and partial balance studies in three others.

Case one, a 70-year-old man, had a ureteral transplantation into the ileum followed for 12 days by continuous suction drainage of gastroduodenal contents. "Adequate amounts of potassium and sodium salts were given." Fever and abdominal distention developed on the seventh postoperative day, together with confusion, belligerence, athetoid and choreiform movements, tremor and muscle twitching. On the eleventh day, the serum magnesium concentration was low (1.46 mEq per liter). When a total of 16 g. of magnesium sulfate was given intramuscularly over three days, the symptoms disappeared within 36 hours. No explanation is given for the serum albumin of 2.0 g. per cent and globulin of 3.6; one wonders if this patient had cirrhosis of the liver.

Case two, a 59-year-old man with decompensated cirrhosis, developed incontinence, confusion, noisiness, restlessness, disorientation, tremor and twitching, progressing to delirium and semi-coma. The manifestations

were relieved 24 hours after a course of magnesium sulfate was begun. The diagnosis of hepatic coma is not discussed.

Case three, a 48-year-old man, had been vomiting for a week, presumbly from pyloric obstruction due to an ulcer of the duodenum. He had severe signs of tetany; a convulsion occurred after intravenous calcium gluconate injection, and severe delirium appeared by the fourth day. Magnesium sulfate was given intramuscularly from the fifth to the fourteenth day. The sensorium cleared considerably on the seventh day, 36 hours after the start of magnesium treatment, and became completely clear on the eighth day. This man had severe alkalosis when he came to the hospital, with decreased serum potassium level, extreme diminution of serum chlorides and a high blood urea nitrogen. Undoubtedly his electrolyte pattern was greatly altered in other ways not measured.

Case four, a confused and hallucinating alcoholic man of 34 years, had a severe hypopotassemic alkalosis. The authors point out that delirium worsened after potassium was given, and tremor and other neuromuscular signs appeared. These improved eight hours after magnesium therapy was started and cleared entirely in 46 hours. The reader is not told the cause of this patient's alkalosis, nor whether he had cirrhosis. Vomiting would scarcely explain the findings; one presumes that the patient was thought to have delirium tremens.

The authors point out that "the development of neuromuscular manifestations in cases three and four when hypopotassemia was being corrected . . . may well be related to the experimental observaton that potassium accentuates the manifestations of magnesium deficiency."

Flink and his colleagues studied the urine excretion of a parenteral dose of magnesium sulfate in three patients with chronic alcoholism, tremor, twitching and mild delirium. They found 35 to 67 per cent in the urine, whereas the normal subject excretes all of the dose in the urine under comparable circumstances.

A number of criticisms may be raised. The observations suggest a clinical syndrome of magnesium deficiency, but lack the dramatic cure one observes with specific magnesium replacement therapy in expermental animals. To the reader, the syndromes of hepatic coma, delirium tremens, hypopotassemia and magnesium deficiency appear insufficiently distinguishable with the evidence at hand. This is not to deny that the patients may have had magnesium deficiency; probably the best evidence that they did is the retention of the element after a parenteral dose.

Magnesium deficiency has been produced experimentally in man by M. G. Fitzgerald and P. Fourman (*Clin. Sci.* **15,** *635 (1956); Nutrition Reviews* **15,** *232 (1957)*), but it is difficult to achieve. Reduction of intake even to 1.1 mEq per day was not enough to cause a large negative balance, so a cation exchange resin was added to the deficient diet of one subject. Even so, the two subjects attained total negative balances of only 46 and 72 mEq respectively in 20 and 24 days (less than 5 per cent of the body's total of approximately 2000 mEq). No change was detected in blood pressure, electrocardiograms, ability to concentrate the urine, or serum magnesium level, even though the urinary excretion after the twelfth day was reduced to 1 mEq per 24 hours.

With large body stores, the availability of bone magnesium to replace tissue losses, and efficient renal conservation of the element, it is clear that the cellular content can be very well protected. Nevertheless, at the end of the study 25 and 42 per cent of intravenous doses of magnesium salts were retained in the two depleted subjects, whereas none was retained in the controls.

J. W. Agna and R. E. Goldsmith have reported three cases of primary hyperparathyroidism with "clinical abnormalities believed to be related to magnesium de-

ficiency" (*New Engl. J. Med.* **258,** *222 (1958)*). All had neuromuscular irritability; in two this subsided after magnesium sulfate therapy. The authors conclude that "the clue to the presence of hypomagnesemia may be the occurrence of tetany, after surgical correction of hyperparathyroidism, that is refractory to the administration of large amounts of calcium." Whether the hypomagnesemia is a primary effect of parathormone or a secondary result from skeletal or soft tissue depletion is not apparent.

Flink and his colleagues have attempted a very difficult task; to define a clinically recognizable syndrome of magnesium deficiency when even its laboratory detection is a matter of inference. The manifestations which the authors "have ascribed to magnesium deficiency in patients can be listed as follows: muscular twitching and tremor ... choreiform and athetoid movements ... rarely, carpopedal spasm and positive Chvostek's and Trousseau's signs, profuse sweating; tachycardia and occasionally, fever; mild anxiety to severe agitation; mild to severe delirium with hallucinations, confusion and disorientation, abusive language and maniacal behavior; finally, convulsions and coma."

Surely a number of etiologically disparate entities share various of these signs. The authors recognize that there may be little correlation between the serum magnesium concentration and the presence of signs or symptoms since serum electrolyte levels are a very poor measure of intracellular concentrations or body stores. This is borne out repeatedly in clinical practice.

ENERGY METABOLISM IN MALNOURISHED INFANTS

Metabolic rates in malnourished infants may be increased per unit of weight because of fat losses. Severe starvation will depress metabolic rate in infants as well as in adults.

After nearly 30 years, the oxygen consumption of malnourished infants has been reinvestigated by F. Varga (*Pediatrics* **23,** *1085 (1959)*). Studies made in the 1920's suggested that a high metabolic rate occurred in infant malnutrition in contradistinction to more recent studies in adults which showed a decreased oxygen consumption on starvation regimes (A. Keys *et al.*, *The Biology of Human Starvation, University of Minnesota Press, 1950*).

Varga reinvestigated this problem in eight infants with pyloric stenosis. By making observations when the infants were both starved and dehydrated, then following hydration, and later during the course of realimentation following specific corrective surgery, the influences of dehydration, starvation and feeding could be determined separately. Since infection did not play a role in the development of starvation in these infants, it had no bearing on their increased oxygen consumption.

While the normal value for oxygen consumption of infants in this age group is 7.1 ml. per kg. per minute, the average oxygen consumption of these infants was 4.9 ml. per kg. per minute. During the first three days postoperatively when the caloric intake began to reach minimal maintenance values of 70 calories per kg. and hydration was normal, the oxygen consumption dropped slightly to 4.6 ml. per kg. per minute. After the fifth postoperative day when the intake of food had returned to normal or above, values of 8.0 ml. per kg. per minute were found. This high oxygen consumption was thought to be due to the loss of fat which had occurred during starvation.

Studies of chronic malnutrition likewise revealed an increased oxygen consumption of 8.2 ml. per kg. per minute, but with a fall to 5.5 ml. per kg. per minute in the presence of semi-starvation.

The above studies suggested an explana-

tion for the discrepancy between studies of infants and more recent studies in adults concerning the basal metabolism in malnutrition. In chronic malnutrition, without gross starvation, the increased metabolic activity of the brain, liver and kidneys (less involved in the process of wasting than the fatty tissue) gave an increased oxygen consumption based on body weight. However when greater degrees of caloric starvation were imposed, the oxygen consumption fell to levels almost as low as in the infants with pyloric stenosis, who almost invariably suffered from severe starvation. In many infants with chronic malnutrition actual starvation may not be present to the degree seen in studies on starvation in adults. Although growth ceases, actual starvation of the remaining tissues may not occur.

Although few studies were made on edematous kwashiorkor patients, it was expected that a low metabolic rate would also occur, probably due to the semi-starvation existing in this condition.

SORBITOL AND SERUM VITAMIN B_{12}

The absorption of supraphysiologic doses of vitamin B_{12} by normal subjects is enhanced by the administration of D-sorbitol, whereas absorption of physiologic doses by pernicious anemia patients is unaffected.

It is known that D-sorbitol has vitamin-sparing action when added to diets of animals (T. B. Morgan and J. Yudkin, *Nature* **180,** *543 (1957)*), and that oral administration of D-sorbitol with a test dose of vitamin B_{12} can enhance the serum concentration of this vitamin in animals (S. M. Greenberg *et al., Nature* **180,** *1401 (1957)*) and in man (B. F. Chow *et al., Am. J. Clin. Nutrition* **6,** *30 (1958)*). Hence interest has been aroused in the mechanism by which D-sorbitol exerts these effects and in the question of whether it affects vitamin B_{12} absorption in pernicious anemia patients. Initial reports have indicated that D-sorbitol is without benefit to pernicious anemia patents (L. Ellenbogen *et al., Proc. Soc. Exp. Biol. Med.* **99,** *257 (1958)*; V. Herbert, *Am. J. Clin. Nutrition* **6,** *547 (1958)*; *Nutriion Reviews* **17,** *188 (1959)*).

Three reports from different laboratories (W. P. Boger, D. S. Brashear and J. J. Gavin, *Am. J. Clin. Nutrition* **7,** *318 (1959)*); Herbert *et al., Ibid* **7,** *325 (1959)* and Chow *et al., Ibid* **7,** *328 (1959)*) confirm and extend the previous observations.

The first of these reports, by Boger *et al.* is concerned with the mechanism of action of D-sorbitol. The observations on animals (Morgan and Yudkin, *loc. cit.*) indicate that D-sorbitol in the diet favors the development of an intestinal flora that synthesizes large amounts of vitamins. The possibility has also been considered that such an effect may contribute to the elevated serum vitamin B_{12} concentration in man following D-sorbitol administration (Ellenbogen *et al., loc. cit.*). To obtain further information about this, Boger *et al.* studied the effect of D-sorbitol alone and together with vitamin B_{12} on serum vitamin B_{12} concentrations.

Three groups of ten subjects each were studied for four periods in an experiment lasting 16 weeks. There was a pretreatment period of four weeks followed by a four-week period during which all subjects were given 20 g. of D-sorbitol daily. During the third period one group continued to receive D-sorbitol while the other two groups received one of two preparations containing 50 micrograms of vitamin B_{12} with 20 g. of D-sorbitol. Finally, there was a post-treatment control period. Venous blood samples were drawn weekly and vitamin B_{12} concentration was estimated by a microbiological assay method using *Lactobacillus leichmannii* as the test organism.

In none of the groups did the administration of D-sorbitol alone for four weeks result in an increase in serum vitamin B_{12}. In fact, on the average, there was a decrease. Further treatment of one group with sorbitol alone for another five weeks resulted in a small but insignificant rise. Treatment of the other two groups with sorbitol and vitamin B_{12} caused a considerably greater rise in serum vitamin B_{12} concentration. When the treatment was stopped, the serum vitamin B_{12} values fell to within the normal range in one week.

The authors emphasize that variability within groups of normal subjects and inherent variability in the assay method make interpretation difficult. However they conclude that D-sorbitol does not act by stimulating the production of vitamin B_{12} by intestinal microorganisms but rather that absorption of the vitamin is facilitated.

It is unfortanate that in no period in this experiment did the subjects receive vitamin B_{12} alone. This would have provided an opportunity to measure the effect of D-sorbitol and vitamin B_{12} separately as well as together, and would have provided a more controlled experiment. Also, there is enough of a trend toward higher serum vitamin B_{12} concentrations after the administration of D-sorbitol alone to warrant a longer examination of this effect and to determine whether D-sorbitol administered with cobalt might not be more effective than D-sorbitol alone.

The second paper by Herbert *et al.* describes a study of the influence of sorbitol on vitamin B_{12} absorption by subjects able to produce intrinsic factor. They used the Schilling test, measuring urinary Co^{58} excretion for 24 hours after the administration of a test dose of labeled vitamin B_{12}. They observed that when supraphysiologic doses of vitamin B_{12} (30 micrograms per day) were administered, there was evidence of improved absorption as the result of the concomitant administration of about 10 g. of D-sorbitol

When they ran similar tests on 12 subjects who received only 2 micrograms of vitamin B_{12} daily, the enhancing effect of D-sorbitol was less clear; although some positive effect was seen in eight of the 12 subjects.

The third paper by Chow *et al.* describes a study of the influence of D-sorbitol on vitamin B_{12} absorption by pernicious anemia patients. They also measured absorption by following urinary excretion of radioactivity for 24 hours after administration of a test dose of 2 micrograms of Co^{60}-labeled vitamin B_{12}. A study of more than 100 healthy subjects showed that the average normal urinary excretion is about 11.0 per cent of the test dose. Twelve pernicious anemia patients, on the other hand, excreted less than 1.5 per cent of the administered dose in the urine, and most of them 0.5 per cent or less. The administration of 10 g. of D-sorbitol with the test dose of vitamin B_{12} increased the absorption significantly in only one of the 12 subjects, but only into the low normal range.

In ten subjects with achlorhydria the administration of D-sorbitol did result in a small but consistent increase in the absorption of vitamin B_{12}. Only 1.2 per cent of a test dose of 50 micrograms of vitamin B_{12} was excreted in the urine when the subjects were given the vitamin alone. This was increased to 2.2 per cent by the concomitant administration of D-sorbitol. The increase was statistically significant but the values were still well below those for healthy persons.

One question about this study cannot be overlooked. Why did the authors use a test dose of only 2 micrograms of vitamin B_{12} in their study of pernicious anemia patients and 50 micrograms when testing patients with achlorhydria? The question is of some pertinence in view of the results of Herbert *et al.*, who could not demonstrate a consistent effect of D-sorbitol on the absorption of a 2 microgram test dose of vitamin B_{12} in normal subjects, whereas they could when the test dose was 30 micrograms.

Nevertheless, there is sufficient evidence

to indicate that D-sorbitol is ineffective as a therapeutic measure in pernicious anemia patients (Chow *et al.*, *Am. J. Clin. Nutrition* **4,** *434 (1956)*; **7,** *325 (1959)*; Ellenbogen *et al.*, *loc. cit.*; Herbert, *Am. J. Clin. Nutrition* **6,** *547 (1958)*).

It would certainly be of interest, however, to follow up these experiments with studies in which various levels of labeled vitamin B_{12} and various levels of D-sorbitol are administered to the occasional pernicious anemia patient who appears to respond to treatment with D-sorbitol. Also, a methodical study of the effect of different levels of D-sorbitol on vitamin B_{12} absorption in normal subjects would be valuable.

CARDIAC PATIENTS IN UNDERDEVELOPED COUNTRIES

Cor pulmonale is more common in India than in the United States while the reverse is true for coronary atherosclerosis. Life expectancy in the United States is more than double that of India.

While in the West we ponder the evils of cholesterol in the blood stream or the deplorable incidence of obesity, the greater half of the world's population hungers for more food. It has become popular to attribute our high incidence of coronary vascular disease largely to our high standard of living, our abundance of meat, egg and milk products. Perhaps we can profit by comparing our own situation with that existing in India, a land of dense population, incredibly impoverished, yet striving to correct the condition which hampers its progress.

Starvation as a clinical syndrome is a curiosity in the United States, since it has all but vanished. We have read of it in Japanese prison camps (*Nutrition Reviews* **5,** *86 (1947)*), and in persons subjected to famine for other reasons (*Ibid.* **6,** *210 (1948)*; **7,** *250 (1949)*). H. L. Taylor and A. Keys studied a group of volunteers who were starved until they lost 24 per cent of their body weight (*Science* **112,** *215 (1950)*), and reported that their metabolic rate was decreased 31 per cent and their cardiac work by half. Moreover, they developed an apathy and physical lethargy which was nearly incapacitating.

Malnutrition is a state of anxiety, hunger and exposure, constantly aggravated by inadequate food and relentless infection, which must lead to disillusionment and despair of a degree unknown to us.

With this in mind, the studies by S. Padmavati of patients with heart disease in India are of interest (*Am. Heart J.* **58,** *418 (1959)*). She calls attention to the apathy which made collection of accurate data so difficult and perhaps inaccurate. However, she was able to obtain figures from a few sources and to evaluate them with proper regard for their significance. The first set of data came from established hospitals in some of the 16 states of India; the second from a survey of selected groups (reliable but incomplete), and the third from records of medical insurance agencies which serve selected social groups.

Comparison of the incidence of deaths from heart disease in hospitals, which was 3 per cent for India and 50 per cent for Western countries, demonstrated the fallacy of statistics. Surely this implies that cardiac patients in India cannot be hospitalized but must live and die in their own communities. For this reason, Padmavati suspected that the incidence of heart disease in India, reported at 1 to 3.6 per cent, was grossly underestimated.

However, she was able to make some valid comparisons for certain groups of cardiac patients. The incidence of various types of heart disease in Delhi from 1951 to 1955 as compared with that in the United States was as follows:

Rheumatic heart disease (39.1 per cent *vs.* 23.5 per cent for New England) was the commonest form of cardiac disorder in

Delhi and reached its greatest incidence between the ages of 20 and 30 years. The author felt that this age distribution was more apparent than real, since patients did not seek medical examination until symptoms became troublesome. Consequently the disorder was not recognized until physical exertion (manual labor in men, pregnancy and delivery in women) precipitated congestive failure.

Although hypertensive cardiovascular disease was slightly less common in India than in the United States (21.1 per cent *vs.* 26.2 per cent for New England), it constituted the second major form of heart disease. Perhaps it is significant that it followed toxemic pregnancies in 35 per cent of the cases.

Cor pulmonale, the third most common form of heart disease in India (16.6 per cent *vs.* 1.1 per cent for New England), afflicted men and women with equal frequency. The duration of symptoms before death was short, and autopsy disclosed advanced emphysema (not tuberculosis) as the principal cause. The author favored the theory that respiratory infections were prevalent and remained untreated due to conditions of poverty and insufficient numbers of physicians.

As might be anticipated in a country whose food supplies include relatively little animal fat and animal protein, and whose populace seldom achieves advanced age, the incidence of coronary atherosclerosis was low (11.3 per cent *vs.* 48.5 per cent for New England). Measurements of the concentration of blood cholesterol revealed the expected lower values, particularly in the poorer socioeconomic groups.

Padmavati concluded that much of the cardiac disease of India was a result of inadequate facilities, insufficient numbers of trained physicians, and meager supplies of drugs and antibiotics. In 1951 the life expectancy of an Indian was 32 years as compared with approximately 68 years in the United States.

However, she did not mention several other factors which might be of equal or greater importance. Inadequate supplies of food, presumably of poor nutritional value, could account for early deaths and the high incidence of infection. Experience in the United States has established the fact that inadequate dietary protein for mothers and children is accompanied by increased rates of toxemic pregnancy and of rheumatic fever in children. Susceptibility to infection is so closely entwined with malnutrition and exposure to the elements, that the combined effect leads to illness and early death.

Although Padmavati intended to portray the inadequacies of medical facilities in her country as the major factor in the production of cardiac disease, she simultaneously supplied evidence of the deleterious effects of inadequate nutrition upon the heart. Those of us who concern ourselves with the alarming incidence of atherosclerotic heart disease in this country might profit from this article. It is evident that India, while suffering little from coronary disease, has a much greater burden to bear, since many of its people do not live to an age at which coronary disease would occur. Meanwhile the factor of "survival of the fittest" has been partially eliminated from the Western scene so that more persons have become eligible for this disorder.

FLUORIDE IN METABOLISM

Kidney changes, including lowered concentrations of fatty acid oxidase, may be of sufficient importance to account for many of the effects observed in fluorotic animals.

Although it is known that two- or threefold increases in protoplasmic fluoride concentrations may be toxic to the actively metabolizing cells of the animal body, the

exact locus of the physiological action of fluoride is not known. Most studies of fluoride have been concerned with its effect upon the skeleton and teeth and its beneficial effect in preventing dental decay, but as early as the turn of the century J. H. Kastle and A. S. Loevenhart (*Am. Chem. J.*, **24,** *491 (1900)*) reported that fluoride inhibited lipase.

R. B. Johnson and H. A. Lardy (*J. Biol. Chem.* **184,** *235 (1950)*) later demonstrated that fatty acid oxidase activity was inhibited by 0.01 molar fluoride. The closely related acetate activating system was inhibited by 0.0001 to 0.0005 molar fluoride *in vitro* (A. C. Aisenberg and V. R. Potter, *Ibid.* **215,** *737 (1955)*). This is within the concentration present in soft tissues under conditions of fluorosis. R. F. Miller and P. H. Phillips (*J. Nutrition* **56,** *447 (1955)*) found that the toxicity of fluoride was enhanced by high levels of dietary fat. This effect was independent of the chain length of the constituent fatty acids.

In a recent study, A. H. Sievert and Phillips (*J. Nutrition* **68,** *109 (1959)*) have examined the *in vivo* effects of fluoride upon fatty acid oxidase reactions, enhancement of fluoride toxicity by dietary fat and the acetylation capacity of tissues in fluorosis.

Weanling rats of the Holtzman strain, weighing between 40 and 45 g. were fed semi-purified diets into which fluoride was incorporated as sodium fluoride. All diets containing increased fat levels were made isocaloric with the normal fat diets by the substitution of roughage for an equivalent of sucrose. At the close of each experiment the animals were sacrified and the tissues needed were quickly excised, chilled and homogenized in isotonic sucrose. Mitochondria were suspended in isotonic sucrose so that the fatty acid oxidase activity could be determined with the Warburg apparatus.

The effects of two levels of fat and two levels of sodium fluoride upon fatty acid oxidase activity in the female rat were studied after the diets had been fed for six to eight weeks. Growth rates were definitely retarded by dietary sodium fluoride and were further depressed by high dietary fat in the fluorotic rat. However, the mitochondrial fatty acid oxidase activity of the livers of these fluorotic rats and of their controls were essentially the same; hence there was evidence of a direct effect of fluoride on this enzyme system. Moreover, the high-fat diet had no influence upon the fatty acid oxidase values, despite its effect of retardation of growth.

G. R. Drysdale and Lardy (*J. Biol. Chem.* **202,** *119 (1953)*) have reported that, although fluoride inhibition of fatty acid oxidation was neglible in a soluble system prepared from rat liver mitochondria, it was complete in a kidney cyclophorase system. The authors point out that since in liver the end product of the oxidation is mainly acetoacetate and since kidney and other organs further metabolize acetoacetate to carbon dioxide and water, the locus of the inhibitory action of fluoride in the fatty acid oxidase system may have occurred at a step beyond the formation of acetoacetate.

The authors, therefore, undertook an experiment to test the effect of sodium fluoride feeding upon kidney fatty acid oxidase. A significant decrease in fatty acid oxidase activity was observable as early as the fourth to fifth day after the beginning of fluoride ingestion. Gross inspection of the kidneys at the four to five day interval indicated that they were unaffected, but chemical analysis showed a highly significant increase in kidney water content. After two weeks on the regimen, the kidneys from the treated animals were yellowish in color, edematous, and presented a granular appearance. Chemical analyses revealed striking decreases in fat and nitrogen concentrations.

The authors are of the opinion that these data did not define the sequence between the enzymatic, chemical and anatomical changes resulting from elevated fluoride ingestion. For example, the striking decrease

in fatty acid oxidase activity could not be accounted for on the basis of the decreased mitochondrial nitrogen content observed in the fluorotic rat kidney.

Since the high fat diet (15 per cent) was also without effect on the liver fatty acid oxidase system, the authors undertook to determine the mode of action of the fat in inhibition of growth. Analysis of fecal materials showed that the fat excretion in fluorotic rats was twice as great as in control rats. Similarly, the nitrogen content per gram of feces was higher in fluorotic rats. Fluorosis in the rat is accompanied by semi-starvation food intakes, but comparison with pair-fed control rats indicated that there was better utilization of the food taken in by the control rats. These results make it evident that it was the fluorosis per se, and not the starvation accompanying it, which was responsible for the altered excretion patterns.

Neither the cause nor the origin of the elevated fecal fat and dry matter observed in fluorotic rats was determined. However, the authors point out that lipid metabolism is intimately associated with coenzyme A and acetyl coenzyme A. One of the functions of coenzyme A in the body is the transfer of acetyl groups. The effect of fluorosis on acetyl transfer was therefore tested, but in none of these experiments was any consistent difference noted in the per cent acetylation of para-aminobenzoic acid by fluoride-fed rats and their pair-fed controls. There was no evidence that the ability of the intact rat to acetylate aromatic amines was inhibited by dietary fluoride.

The authors conclude that the kidney changes, including lowered concentrations of fatty acid oxidase, lipids and nitrogen, may be of sufficient importance and magnitude to account for many of the effects observed in fluorotic animals. The decrease in kidney fatty acid oxidase activity, whatever its cause, must seriously interfere with the animal's ability to metabolize fat.

It should be emphasized that these interesting results on tissues from fluorotic animals have nothing to do with the favorable effects of minute concentrations of fluoride in lessening dental decay.

CHEMISTRY OF INFLAMMATION AND REPAIR

Chemical analysis of skin, injured by croton oil injections, for nitrogen, hexosamine and hydroxyproline were correlated with the histological changes in the same tissue.

The histological description of tissue inflammation and its repair have been known for some time. With the development of chemical procedures, studies have been undertaken to correlate the changes seen on histological examination with the chemical alteration going on within the tissue. Recently, R. Jacob and J. C. Houck (*Surg. Gynec. Obstet.* **109,** *85 (1959)*) have studied the chemical events induced by a croton oil inflammation. Using semi-micro procedures, these investigators determined the nitrogen, hexosamine and hydroxyproline content of the skin of animals before and following the production of a croton oil induced inflammation. The analysis of these three substances, the authors believed, would give them some index of the granulation tissue formation (hexosamine), collagen (hydroxyproline) and tissue protein (nitrogen) in the skin.

For this study 60 male rats weighing between 220 and 250 g. were used. The animals received intradermally 0.4 ml. of croton oil in the right dorsal area. A corresponding area on the other side of the body was used as a control. At various times ranging from three to 21 days following injury, groups of six animals were sacrificed and both the injured and uninjured areas were studied for histological and chemical changes. There was usually a necrotic re-

sponse to the croton oil within 24 hours after injection, and the ulcerous lesion covered an area of about 1.5 sq. cm. Seven days later an eschar formed which thickened during the following days and sloughed on the average of 17 days post-injury. Twenty-four days after the injury, the wound was healed and overgrown with hair.

To negate the influence which variations in the water and fat content of the skin would have upon the chemical content of the various substances studied, these investigators expressed all values as micromoles of hexosamine or hydroxyproline per millimole of nitrogen. Expressed in this manner, there appears to be no statistical difference in samples obtained from various areas of the skin.

In the injured areas of skin, it was found that there was a marked decrease in the ratio of hydroxyproline to nitrogen beginning about the third day following injury. The levels then remained constant until about the eighteenth or nineteenth day post-injury when the hydroxyproline-nitrogen ratio gradually increased, reaching a pre-treatment value by about the twenty-fourth day. The reduction in the hydroxyproline-nitrogen ratio amounted to approximately 30 per cent of the pre-injured value. There were also decreases in the hydroxyproline-nitrogen ratio of the non-injured areas and this followed the pattern of the injured skin, though much smaller in magnitude, having a maximum decrease of only about 15 per cent of the pre-treatment value.

In contrast to changes in the hydroxyproline, the ratio of hexosamine to nitrogen in the tissue increased following injury, reaching a maximum at about the third day. The ratio then decreased rapidly and reached a level approaching the pre-injury value at the time the eschar was forming. From then on, except for minor fluctuations, the values remained slightly elevated above the pre-injury level until about the twenty-fourth day following the induction of the inflammation.

The decrease in hydroxyproline by the third and fourth day post-injury was interpreted as indicating a marked loss of collagen of the skin. With this large breakdown of collagen, one might expect a release of hydroxyproline from the collagen and an increased level in the blood. Such an increase was found with one peak level occurring at the third or fourth post-injury day and a larger increase (seven times normal) occurring on the sixth or seventh post-traumatic day.

The increased hydroxyproline levels which occurred in the serum following the injury were not due to the free amino acid, but believed to be caused by hydroxyproline bound in a large molecular group such as a polypeptide. This explanation was based on the fact that the ratio of hydroxyproline to nitrogen in the serum was similar whether the determination was made on pooled serum or whether it was carried out on protein precipitates produced by adding excess amounts of ethyl alcohol.

These investigators believe that the increase in tissue hexosamine corresponds with the histological changes in which granulation tissue formation is occurring. The decrease in hydroxyproline can be correlated to the histological picture where there is a decrease in tissue collagen. However, at the time when there was little collagen in the wounded area, 70 per cent of the initial hydroxyproline remained in the tissue. This the investigators believed was associated with the reticulin component.

The change in hydroxyproline content of the tissue was interpreted to be an actual decrease of hydroxyproline, since the concentration of nitrogen ranged only from 3.3 to 3.6 millimoles per gram of fresh skin while the hydroxyproline decreased 260 to 300 micromoles per gram of skin. No reason was given why there was a decrease in the hydroxyproline content of the skin on the uninjured side.

Since neither croton oil nor any active fraction of the oil is water soluble, the in-

vestigators do not think there is a systematic effect of the vesicant on the dermal collagen. Nevertheless, at least in the case of collagen formation, there appeared to be a generalized reaction to croton oil injections since there were decreased hydroxyproline levels in the uninjured skin.

These investigators correlated the histological changes with the chemical alterations noted in skin samples of animals having an experimentally induced chemical inflammation. While studies on the collagen, protein, and granulation tissues were possible, future investigations will undoubtedly cover more parameters involved in this problem, including changes in enzyme activity.

RELATION OF WATER AND FOOD INTAKES

Adult dogs maintained in a controlled environment consumed very constant amounts of water each day as long as they were in weight equilibrium. The water intake was directly proportional to the quantity of food and/or salt consumed.

A relationship between the consumption of food and the intake of water has been recognized for many years. J. L. Strominger (*Nutrition Reviews* **15,** *123 (1957)*) showed that in rats the water intake is closely related to the amount of food consumed. In his rats, the water consumption was approximately twice the weight of the dried food consumed. This relationship held even in the rats that had been made hyperphagic by operative lesions in the hypothalamus. Subsequent to this work, H. M. Bruce and G. C. Kennedy (*Nutrition Reviews, loc. cit.*) found that the water intake was influenced by the level of protein in the diet. It was postulated that the higher water consumption of the rats fed the high protein diets was related to the water needed for renal clearance of the nitrogen compounds (*Nutrition Reviews* **13,** *157 (1955)*).

L. J. Cizek (*Am. J. Physiol.* **197,** *342 (1959)*) maintained adult dogs on measured food and water intakes for periods as long as six months. A dry commercial diet containing 26 per cent protein, 10 per cent fat, and 8.5 per cent minerals was given to the dogs in amounts required to maintain them at constant weight. The diet was mixed with an equal volume of water. The water consumed during each 24-hour period was measured, allowing for that lost by evaporation. Cizek (*loc. cit.*) points out that dogs usually drink promptly after eating, and this represents a major portion of the water intake.

Daily water consumptions for one dog over a six-week period showed a range from 18.2 to 35.0 ml. per kg. of body weight. In spite of this marked variation in the daily consumption, weekly averages for this dog ranged from 23.7 ± 4.6 to 27.4 ± 4.4. There were only slight variations in the body weight of the animal throughout this period. Consequently, the fluctuations in water consumption were due to some other factor.

The variation in water consumption over a six-week period was considerably less among other dogs. For instance, one dog showed a day-to-day range from 25.4 to 27.5, with an average of 26.3 ml. per kg. of body weight. The constancy of water intake when the food consumption remained the same was maintained in some adult animals for periods as long as four years.

In spite of the constancy of the individual water consumption, there were marked variations between individual dogs. This was shown in the average daily water consumption of 12 adult dogs (six males and six females) fed the same diet in amounts that maintained their weight. The average daily water consumption over a six-week period ranged from 22.5 to 35.7 ml. per kg. for the different dogs.

In another series of experiments, 100 g. of the dry stock diet was mixed with ¼, ½, ¾,

1, 1½, 2, 3, 4 or 5 parts of water, and each water-diet mixture was fed daily for ten days. As the water content of the diet increased, the amount of water consumed apart from that in the diet mixture decreased. The reduction was such that the total amount of water remained constant until the diet containing 2 parts of water was fed. Thereafter, however, continued addition of water to the food caused no further decrease in drinking response so that the total water consumption began to increase.

The addition of salt to a constant amount of dry commercial diet resulted in an increase in water consumption. This was proportional to the amount of salt added, which was from ½ to 25 g. per day. It may be assumed that the dogs were maintained on each salt level for ten days since Cizek stated (*loc. cit.*) that all "observations be done in multiples of ten before any of the material was to be used as adequate and reliable evidence." The dogs tolerated even the highest level of salt and showed "no untoward physiological effects."

Another series of experiments demonstrated that the water consumption of the dogs was always proportional to the amount of dry food consumed. There was, in fact, a straight line relationship between water consumption and the weight of food when this ranged from 0 to 500 g. per kg. of body weight, and this linear relationship was maintained even though the water content of the diet was altered. This again indicated the accuracy with which the dog adjusted the water it drank so that the total water intake remained constant for a specific weight and type of food.

It was also found that in the total absence of food, the dogs reduced their water consumption. While the water consumption remained fairly level at 25 ml. per kg. of body weight during the control week when the dogs received a constant weight of food, as soon as the food was removed the water consumption dropped to about 15 ml. for the first day or so. It then gradually increased until toward the end of the week it was 20 ml. When food was again given to the animals after the one-week starvation period, the water consumption immediately rose to 39 ml. and then gradually decreased during the remainder of that week until it reached the pre-starvation level.

Guinea pigs and rabbits are exceptions in the preceding reaction to food deprivation. Earlier work by Cizek (*Am. J. Physiol.* **179,** *104 (1954)*) indicated that when all food, but not water, was removed from guinea pigs, they developed an extreme polydipsia which led to a mortality of 50 per cent of the animals by the third day of starvation and 70 per cent by the fifth day. "The animals develop severe quivering, rear up on their haunches and then topple over" with death occurring during a convulsive seizure. When guinea pigs were deprived of both food and water, there was no mortality during the five-day study.

Rabbits deprived of food, but permitted water, also developed polydipsia but showed no neurological symptoms. However, some rabbits in the course of a 13-day starvation period became edematous (H. L. Rosenthal and L. Cravitz, *J. Nutrition* **64,** *281 (1958)*). It of interest that this development of edema during the withholding of food does not occur in other animals (A. Keys *et al.*, *The Biology of Human Starvation, Vol. 2, p. 921. Univ. of Minnesota Press, Minneapolis, 1950.*)

There is no explanation for the unusual behavior of guinea pigs and rabbits deprived of food. Those guinea pigs that survived the starvation period with unlimited water intake showed no apparent change in the percentage of total body water present in the gastrointestinal tract; it was 17 per cent for both males and females as compared with 17 per cent for males and 20 per cent for females receiving food and water ad libitum (Cizek, *Am. J. Physiol.* **179**, *104 (1954)*).

Cizek has suggested that the explanation for the relation between water and food consumption may lie in the osmotic pressure of the diluted food contents in the gastrointestinal tract (*Am. J. Physiol.* **197,** *342 (1959)*). In addition, the requirement for renal excretion as influenced by the composition of the diet may also play a role.

All animals in the above studies were maintained under standardized laboratory conditions. Consequently, the fluctuations in water consumption that can result from variations in activity and external temperature were eliminated.

These observations on the relationship between water intake and food consumption accentuate the hiatus in our knowledge concerning the factors that control both thirst and appetite at such constant levels. They emphasize very vividly the fact that when food consumption remains constant, the water intake is regulated with considerable constancy and precision. It will remain for future research to explain the mechanisms involved in these phenomena.

IN VITRO ENHANCEMENT OF TISSUE UPTAKE OF VITAMIN B_{12} BY INTRINSIC FACTOR

Uptake of vitamin B_{12}-Co^{60} by rat liver slices and small intestine is enhanced by incubation with hog intrinsic factor concentrate in the presence of calcium. This permits quantitative studies and in vitro assay for intrinsic factor.

W. B. Castle's original pernicious anemia hypothesis, that extrinsic factor interacts with intrinsic factor to form the "antipernicious anemia principle", has been modified with the finding that vitamin B_{12} (cyanocobalamin) is both extrinsic factor and antipernicious anemia principle. Thus the function of intrinsic factor apparently is to facilitate the absorption of cyanocobalamin and possibly also to form a more potent complex. The evidence is against the latter.

The binding of vitamin B_{12} by gastric juice has been reviewed (*Nutrition Reviews* **15,** *90, 179 (1957)*). However, many other substances bind the vitamin, and assays based on this principle are therefore not valid. Intrinsic factor concentrates have been made with activity in milligram amounts; the activity is evidently quite specific, for gastric juice does not increase the absorption of folacin or carbohydrate or protein in pernicious anemia.

There are observations suggesting that intrinsic factor may prepare the intestinal mucosa for the absorption of cyanocobalamin. The relation between the two substances is not stoichiometric. Accumulating evidence suggests that intrinsic factor may be a mucoprotein or mucopolypeptide. One of the difficulties in purifying and studying this material has been the lack of a simple assay for intrinsic factor. Currently used assays are based on the effect of intrinsic factor concentrates on the absorption of radioactive cyanocobalamin in man or animals (*e.g.* rats). This has supplanted the classical "reticulocyte response" method to a large degree.

However, there is accumulating evidence of species differences in several respects. Patients have been described who became refractory to hog pyloric mucosa or hog intrinsic factor concentrate, and who still responded to human gastric juice. The subject of refractoriness has been previously reviewed (*Nutrition Reviews* **16,** *235 (1958)*). Another review (*Ibid.* **16,** *307 (1958)*) discusses "intrinsic factor and absorption of vitamin B_{12}" and some of the differences between the action of hog, rat and human intrinsic factor in the rat and in man.

The question of species specificity is further complicated by the presence of various binding substances, including excess

intrinsic factor itself, in almost any preparation of intrinsic factor (see below).

Since vitamin B_{12} is effective locally when injected into the marrow, and since intrinsic factor has seemed so obviously concerned with absorption, there has been little effort to find out whether the latter has any tissue effects. O. N. Miller and F. M. Hunter (*Proc. Soc. Exp. Biol. Med.* **96,** *39 (1957)*) showed that hog intrinsic factor concentrate increased the uptake of Co^{60}-labeled cyanocobalamin by rat liver slices.

V. Herbert has pursued this line of study and published several papers on the subject. One is entitled "Studies of the Mechanism of the Effect of Hog Intrinsic Factor Concentrate on the Uptake of Vitamin B_{12} by Rat Liver Slices" (*J. Clin. Invest.* **37,** *646 (1958)*). The liver slices were incubated in Hastings' bicarbonate buffer to which hog intrinsic factor concentrate and Co^{60}-labeled vitamin B_{12} were added in fixed amounts. Control experiments were run with hog intrinsic factor concentrate replaced by saline. The results were expressed as retained radioactivity of washed slices (counts per minute per gram of liver slices).

Herbert found as much as ten times the uptake of Co^{60}-labeled vitamin B_{12} by liver slices incubated with hog intrinsic factor concentrate as by the control slices. The buffer used was not important; 0.9 per cent sodium chloride containing calcium chloride was as good as any. Enhancement of uptake by the intrinsic factor concentrate was abolished by heating the liver slices or heating the concentrate. Dinitrophenol did not diminish the enhancement effect. Uptake in the presence of the intrinsic factor increased with time for the first hour, leveling off after three hours or more. The enhancement effect was comparable at 3°C and at 37.5°C.

Replacement of various buffer ions, except for calcium, had no effect on enhancement. When this ion was replaced by an equimolar concentration of potassium, the intrinsic factor concentrate decreased uptake (40 counts per minute compared with 390 for the control slices). A number of ions, including magnesium, were not effective substitutes for calcium but strontium was. To test the effect of a chelating agent, standard one-hour incubations were performed, followed by a second one-hour incubation with added sodium chloride or ethylenediaminetetraacetate.

Ethylenediaminetetraacetate eluted no more radioactive material from the control slices than did sodium chloride. However, most of the labeled vitamin taken up by slices incubated with the concentrate could be removed by ethylenediaminetetraacetate. In other experiments, no enhancing effect was observed when binding substances such as heparin, chondroitin sulfate or orosomucoid were used in place of the intrinsic factor concentrate.

Later work by Herbert (but published earlier) is entitled "Development of a Possible *in vitro* Assay for Intrinsic Factor" (*Proc. Soc. Exp. Biol. Med.* **97,** *668 (1958)*). He used rat liver slices in Krebs-Ringer solution with 0.1 molar tris-hydroxyaminomethane buffer and calcium chloride added to a standard concentration. Determinations were made at 0°C, as enhancement was more striking at this temperature; in addition, the slices did not become friable in the cold. The Co^{60}-labeled cyanocobalamin was added in fixed amounts and the amount of the intrinsic factor concentrate varied. The two substances were added simultaneously before incubation, or in sequence (one hour's incubation followed by washing and a second hour with the second mixture). The results were again expressed as retained radioactivity of washed slices.

An optimal concentration of the intrinsic factor concentrate was found; greater or lesser concentrations resulted in less enhancement of cyanocobalamin uptake in the simultaneous incubation system. The most striking finding was that liver slices incubated for one hour with the concentrate and a second hour with labeled vitamin B_{12} took up much more than slices incubated in the two materials together. After simulta-

neous incubation, a second hour with the vitamin increased the uptake to levels found in sequential incubation. When different preparations of the intrinsic factor concentrate were used with sequential incubation, the retained radioactivity correlated well with relative potencies as assayed by Schilling's urinary excretion test.

Gastric juice from two pernicious anemia patients had no effect on uptake of labeled cyanocobalamin; juice from a patient with nutritional vitamin B_{12} deficiency and one with malabsorption syndrome did enhance the uptake. All four patients had histamine-fast achlorhydria. Neither heparin nor chondroitin sulfate enhanced uptake; the incubation system is therefore not a measure of binding per se.

The author recognizes that inhibitors or competitors of intrinsic factor may be present in the concentrates used; he points out that despite these possibilities his findings may well serve as an *in vitro* assay for intrinsic factor.

Herbert interprets his findings as suggesting that the hog intrinsic factor concentrate enhances uptake by physical rather than metabolic means, and that the action is not a nonspecific mucopolysaccharide or mucoprotein effect. He derives a working hypothesis, that intrinsic factor has free binding sites which attach to receptor sites on liver (in the presence of calcium), leaving other sites on the intrinsic factor molecule free to bind vitamin B_{12} (see below).

This hypothesis is further elaborated in a later paper by Herbert entitled "Mechanism of Intrinsic Factor Action in Everted Sacs of Rat Intestine" (*J. Clin. Invest.* **38**, *102 (1959)*). The same mechanism appears to operate in the small intestine and in the liver slices.

The experimental protocols were the same as those used in the previous work with liver slices. The small intestine from freshly sacrificed rats was turned inside out, washed in ice-cold 0.9 per cent saline solution and cut into segments, each tied at both ends to avoid serosal vitamin B_{12} uptake. Incubations were performed at 6°C.; the results were "strikingly similar" to those of the experiments with rat liver slices.

Simultaneous incubation showed no enhancement of cyanocobalamin uptake by the hog intrinsic factor over a 40-fold range of concentration. Enhancement was marked with incubation in sequence (first with the concentrate, then with vitamin B_{12}-Co^{60}) but there was little or none when tenfold or 100-fold higher or lower concentrations of the intrinsic factor were used.

Thus, both in the rat liver and small intestine experiments, enhancement of vitamin B_{12}-Co^{60} uptake was most marked with sequential incubation; it also took place in the cold, was favored by calcium and was partially reversible by later incubation with ethylenediaminetetraacetate. No enhancement was observed in calcium-free media in the case of liver, but some did occur in the case of small intestine. The author speculates that this may have been due to the difficulty of washing the intestinal mucosa free of calcium.

In other experiments on small intestine segments, the enhancing effect of the intrinsic factor concentrate on the vitamin uptake was abolished by preheating the concentrate at 100°C or by incubation at pH 1.85. 5,6-Dimethylbenzimidazole, with or without cobaltous chloride, did not affect vitamin B_{12}-Co^{60} binding. The author infers that these portions of the cyanocobalamin molecule may not be involved in the interaction with intrinsic factor.

The similarity of Herbert's results in the liver slice and small intestine experiments suggested to him that intrinsic factor had the same action in both loci. He explains his working hypothesis schematically, visualizing the intrinsic factor molecule as a midpiece which, on one side, locks into receptor sites on liver or intestine in the presence of calcium. On the other side of the molecule is a (different) receptor site for cyanocobalamin, the tailpiece. "Possession of these two properties simultaneously may be unique to intrinsic factor. Heparin, chondroitin sulfate

and orosomucoid do not increase vitamin B_{12}-Co^{60} uptake" by either small intestine or liver slices, indicating that "enhancement of vitamin B_{12} uptake is not a nonspecific property of any mucopolysaccharide or mucoprotein."

The author pictures the "ideal" situation in which there would be identical numbers of receptor sites, intrinsic factor molecules and vitamin B_{12}-Co^{60} molecules so that maximal absorption of the vitamin would take place. If too little intrinsic factor were present there would be less cyanocobalamin uptake. To explain the phenomenon of decreased vitamin B_{12} absorption in the presence of an excess of intrinsic factor *in vivo*, Herbert visualizes some liver receptor sites occupied by intrinsic factor to which no vitamin is attached, while the incubation medium still contains cyanocobalamin attached to intrinsic factor molecules for which no mucosal site is available. Thus all intestinal receptor sites would be occupied, but not all by vitamin-binding intrinsic factor.

Incubation in sequence would bind intrinsic factor to liver or small intestine and, after washing, none would be left for the cyanocobalamin to attach to except that already bound to liver or small intestine. Not all of the facts are explained by this theory, *e.g.*, lack of enhanced vitamin uptake by small intestine after sequential incubation with large amounts of hog intrinsic factor concentrate. Herbert speculates that the intrinsic factor may be trapped between villi and later released "to compete for the vitamin with the intrinsic factor on receptor sites."

Although one could object to Herbert's theory, he finds it helpful (when applied to intestinal absorption) in explaining a number of disputed points about species specificity. Since intrinsic factor preparations are generally contaminated with other binding materials, vitamin B_{12}, in any simultaneous incubation experiment, distributes itself between intrinsic factor and the other binding materials. A large enough excess of otherwise potent hog intrinsic factor concentrate can bind all the receptor sites, as explained above, leaving a free excess to compete for cyanocobalamin and make it non-absorbable, whereas a lesser dosage of intrinsic factor preparation or a later dose of vitamin B_{12} might allow absorption of the vitamin. Herbert reasons that if too much intrinsic factor can saturate the cyanocobalamin, there is considerable doubt whether species specificity is important. "Finally, the demonstration that hog intrinsic factor concentrate will enhance vitamin B_{12} uptake by rat small intestine further weakens the . . . concept of species specificity of intrinsic factor as a phenomenon of major importance."

Herbert has performed a valuable service in investigating the action of intrinsic factor in the liver and will broaden our outlook, since most investigators have assumed that this substance acts only at the intestinal absorptive epithelium. If the liver is a physiologic site, this is useful information. If not, then comparison with similar experiments on the intestine should furnish significant differences with which to improve our understanding of the physiology of intrinsic factor. Herbert's theory will be a stimulus to further work.

EFFECT OF CARBOHYDRATE SOURCE ON CHOLESTEROL AND CARDIOVASCULAR SYSTEM

Rabbits fed lactose and cholesterol developed significantly more aortic atheroma, with higher serum and hepatic cholesterol levels, than did rabbits fed sucrose and cholesterol.

Increasing numbers of nutritionists, experimental and clinical alike, have for some time now discarded the once current but unfortunate concept of dietary carbohydrate

as only supplying caloric needs (so-called "empty calories"). Evidence is now abundantly at hand to indicate that the kind of carbohydrate may have profound influences on protein and lipid metabolism in both health and disease (M. W. Marshall and M. Womack (*J. Nutrition* **52,** *51 (1954)*; G. E. Peterson, E. C. Dick and K. R. Johansson, *Ibid.* **51,** *171 (1953)*; *Nutrition Reviews* **13,** *23, 25 (1955)*; **17,** *93 (1959)*).

Two years ago dietary lactose was found to increase the intestinal absorption of cholesterol in the rat (W. W. Wells, *Fed. Proc.* **16,** *402 (1957)*; Wells and S. B. Cooper, *Arch. Biochem. Biophys.* **75,** *273 (1958)*). This group (Wells and S. C. Anderson, *J. Nutrition* **68,** *541 (1959)*) has now taken a logical step forward in these investigations by contrasting the atherogenic effect of lactose with that of sucrose (29.35 per cent) in diets of rabbits supplemented with 0.35 per cent cholesterol (18 animals in each group). Pathologic lesions were correlated with levels of cholesterol in the animals' sera and livers. These various food mixtures all contained cottonseed oil (13 per cent) and cod liver oil (2 per cent) as the dietary fat. Soybean meal (45 per cent) was used in place of casein as the source of protein because it had been found previously that rabbits of both groups consumed the diet best when soybean was used. All animals gained weight under these conditions, those receiving the lactose-containing diet to a somewhat lesser degree than those in the sucrose-fed group.

Despite this lesser degree of weight gain by animals in the lactose group, significantly more severe degrees of atheroma were found on gross examination of their aortas seven to eight weeks after initiation of these dietary regimens than were present in animals in the sucrose group. Measurements of surface areas of intimal lesions by three independent observers revealed that changes in the vessels of the lactose-fed rabbits were five times as extensive as those in the aortas of rabbits fed sucrose. Cholesterol contents of livers and sera in the latter were, as might be expected, significantly lower than in the rabbits on the lactose-containing diets.

The authors point out that the increased atherogenesis in the lactose group could not be ascribed to a higher absolute intake of cholesterol because this group actually consumed 2 to 4 g. less of the diets daily than did the rabbits in the sucrose group. Differences between the two groups in the amounts of cholesterol in sera and livers as well as in the relative severity of aortic atheromatous changes were far too great to be ascribed to either physiologic or chance variation.

Intestinal motility was shown, by a special technique, to be greater in the lactose-fed animals than in the sucrose controls, although diarrhea was absent in both groups. Therefore, the greater absorption of cholesterol from the intestines of the lactose-fed animals could not be attributed to lesser motility. The authors suggest that a contaminant of the lactose used (possibly lactaminic acid) might have increased cholesterol absorption, and they are repeating their study using a purified lactose. We hope it will be possible to measure cholesterol absorption more directly in these new experiments.

In suckling rabbits levels of serum cholesterol are elevated (J. H. Bragdon, *Circulation* **5,** *641 (1952)*), and chickens fed sucrose-cholesterol diets develop higher cholesterol levels in both sera and livers than do chickens fed glucose-cholesterol food mixtures (D. Kritchevsky, R. R. Kolman, R. M. Guttmacher and M. Forbes, *Circulation* **20,** *964 (1959)*). There are old reports of high incidences of atherosclerosis in certain primitive peoples subsisting on diets containing large amounts of milk (B. Kuczynski, *Klin. Wochschr.* **4,** *39 (1925)*). Many of our own American population, while not primitive, should also be regarded as heavy milk drinkers. There is a recent report, based on autopsy data, that the incidence of coronary arterial thrombosis and myocardial infarction in patients with peptic ulcers treated with Sippy diets was twice that found in a group of selected control subjects with peptic

ulcers not subjected to this therapy (W. A. Thomas *et al.*, *Circulation* **20,** *992* (*1959*)).

Whether or not such random observations in man can be related to the experiments of Wells and Anderson (*loc. cit.*) cannot be decided now. However, there are sufficient data at hand to warrant active pursuit of these leads in future experimental and epidemiologic attacks on cardiovascular disease. It would be of interest to know whether intakes of animal carbohydrate parallel those of saturated fats in populations with high or low susceptibilities to myocardial infarction. There should be a search for other nutrients related to heart disease in addition to those associated with fats and sterols.

MUSCLE IN VITAMIN E DEFICIENCY

Although various changes in dystrophic muscle are observed on vitamin E-deficient diets, these changes fail to show direct correlation with muscular dystrophies of different origin.

Although muscular degeneration is observed in chicks fed diets low in vitamin E, sulfur amino acids and selenium (H. Dam *et al.*, *Acta Pathol. Microbiol. Scandinav.*, **31,** *172* (*1952*); Dam and E. Sondergaard, *Experientia* **13,** *494* (*1957*)), little is known of the biochemical alterations actually occurring in the muscle. According to Dam *et al.*, the degeneration in the skeletal muscles is accompanied by reduced amounts of creatine and increased levels of cholesterol.

These investigators also described the histological changes in chick muscle which included perivascular infiltration with so-called "round" cells and stated that the changes resembled those described in muscular dystrophy from dietary causes in guinea pigs, rabbits and ducklings (M. Goettsch and A. M. Pappenheimer, *J. Exp. Med.* **54,** *145* (*1931*); **59,** *35* (*1934*)). Furthermore, studies on dystrophic muscles in man and in mice with hereditary muscular dystrophy have shown lowered activities of the phosphorylating enzymes (J. C. Dreyfuss *et al.*, *J. Clin. Invest.* **33,** *794* (*1954*)); S. L. Leonard, *Proc. Soc. Exp. Biol. Med.* **96,** *720* (*1957*)).

All these effects have recently been the subject of an extensive study by M. C. Nesheim, Leonard and M. L. Scott (*J. Nutrition* **68,** *359* (*1959*)). Levels of glycogen, phosphorylase, total dry matter, ash, sodium, potassium and creatine were ascertained in skeletal muscle of control and vitamin E-deficient chicks.

In three experiments, nearly all chicks on the basal vitamin E deficient diets showed grossly visible evidence of muscle damage similar to that reported by Dam *et al.* (*loc. cit.*), whereas no muscle damage could be seen in chicks receiving vitamin E. The breast muscles seemed to be most affected. Lesions appeared first at this site and also involved a larger proportion of the muscle fibers than in the legs. Histological examination of the dystrophic muscles showed definite degenerative changes with marked cellular infiltration as described above.

In the first experiment, in which a Torula yeast-containing diet was used, the chicks also showed symptoms of exudative diathesis. This was not observed in the second and third experiments in which the chicks were fed a casein-gelatin diet. However, the muscular dystrophy in the chicks receiving the different basal diets was similar in all characteristics measured.

A significant decrease (on a wet weight basis) occurred in both the active phosphorylase and total phosphorylase in white (or breast) muscle. A lesser, but nonetheless significant, decrease was observed when the observations were expressed in terms of dry weight. The active and total phosphorylase activities in white muscle were twice that found in red muscle in both

experimental and control animals. The ratios of active to total phosphorylase in the dystrophic and normal muscle were similar, as were the ratios in white and red muscle. Glycogen levels were higher in white muscle.

The muscle of chicks receiving the basal diet contained less dry matter than the muscles of chicks receiving vitamin E. Changes in potassium and creatine content of dystrophic muscles were variable. The authors explained decreases in these components as functions of increased water content in the muscles.

This is in contrast to the relatively large decrease in creatine observed earlier in other animals (Goettsch and E. F. Brown, *J. Biol. Chem.* **97,** *549 (1932)*; K. L. Blaxter and R. F. McGill, *Vet. Rev. Annot.* **1,** *91 (1955)*). Creatine was significantly lower in the dystrophic muscles, as expressed on a wet basis only. The sodium content of the breast muscles of vitamin E-deficient chicks was higher in terms of both wet and dry weight. An increase in total ash in the vitamin E-deficient chicks was not statistically significant.

It is the role of phosphorylase in the synthesis and degradation of muscle glycogen that warrants a study of this enzyme in conditions of muscular dystrophy. The authors note that the activity ratios of phosphorylase were similar in normal and dystrophic muscle. Thus, the phosphorylase *a* remains proportionately constant. This situation prevails in mice with hereditary muscular dystrophy. Other studies, however, indicate that changes in phosphorylase *a*, which, of course, result in ratio alterations, can be brought about by extensive contraction of muscle.

The authors point out that, although in the case of vitamin E deficiency it might seem logical to relate the changes in phosphorylase to muscle glycogen levels, the situation does not hold for the hereditary dystrophic mice. In the latter, changes in phosphorylase parallel those found in the vitamin E-deficient chicks. Yet the concentration of glycogen in the dystrophic mouse muscles was higher than in the controls. Thus it is not possible to conclude that all muscular dystrophies are caused, directly or indirectly, by common deficiency states.

LINOLEIC ACID AND CHOLESTEROL METABOLISM

The addition of linoleic acid to a stock rat diet increases the incorporation of radioactive carbon in liver cholesterol and increases the excretion of the components of cholesterol catabolism.

The ingestion of unsaturated fats causes a fall in serum cholesterol in man while saturated fats may give rise to elevation of serum cholesterol.

In order to gain information about the mechanism of the above effect, J. M. Merrill (*Circ. Res.* **7,** *709 (1959)*) determined the effect of feeding saturated and unsaturated fatty acids on the incorporation of 1-C^{14}-acetate into liver cholesterol and the effect on excretion of cholesterol metabolites.

Male Sprague-Dawley rats were fed a stock diet with 10 per cent commercial linoleic acid or coconut oil added, or stock diet alone. After eight to 19 days, 6 microcuries per 100 g. body weight of 1-C^{14}-sodium acetate were injected 30 minutes before death. Livers were extracted for three minutes with chloroform in a tissue shredder at 14,500 r.p.m., filtered and re-extracted. The filtrates were evaporated in an air stream and cholesterol was precipitated as digitonide. After repeated washing and centrifuging, the purified dried cholesterol digitonide was weighed and an aliquot counted in a windowless flow counter.

For comparison with the *in vivo* experiments, liver slices of rats receiving the various diets were incubated with 12.5 microcuries of sodium acetate-1-C^{14} in Krebs-Ringer phosphate buffer at 37°C and 100 per cent oxygen atmosphere. Cholesterol was isolated and radioactivity determined.

Excretion of metabolic products of cholesterol metabolism was determined on frozen, homogenized 1 g. samples of feces taken from 24-hour collections in each of three groups of animals. After chloroform extraction, aliquots were analyzed for Lieberman-Burchard chromogens, 3-beta-hydroxy sterols and bile acids.

In the *in vivo* experiments, an increase of 259 per cent in radioactivity of liver cholesterol occurred with the addition of linoleic acid to the diet.

By sacrificing animals at 15, 30 and 45 minutes after injection of radiocarbon and analyzing the liver cholesterol, it was possible to compare the rate of incorporation of radioactive carbon in cholesterol. In rats fed a stock diet with added linoleic acid, the radioactivity reached a peak in 15 minutes and fell in the next 30 minutes. Radioactivity of cholesterol from rats fed the stock diet alone did not reach a peak until 30 minutes following injection.

Determination of radioactivity of cholesterol in animals sacrificed 120 minutes after injection showed 2.87 counts per second per mg. in control samples compared with 3.97 counts per second per mg. in linoleic-fed animals. Similarly, in animals sacrificed 240 minutes after injection the control cholesterol radioactivity was 3.0 counts per second per mg. compared with 5.5 counts per second per mg. in linoleic acid-fed animals. These results were not statistically significant.

When comparing animals receiving saturated fat with control, stock diet-fed animals, the cholesterol digitonide radioactivity of the stock animals after eight days was 1.52 counts per second per mg. compared with 1.09 counts per second per mg. for the coconut oil group. Thus specific activity of liver cholesterol was decreased by about 30 per cent in animals receiving the saturated oil supplement.

The *in vivo* observations were confirmed by *in vitro* studies of incorporation of radioactive carbon into cholesterol. After one week on linoleic acid or coconut oil-supplemented stock diets, animals were sacrificed and liver slices incubated and analyzed. In animals ingesting linoleic acid the radioactivity was 71 per cent higher than in animals eating the diet with added coconut oil.

Average free cholesterol for stock diet animals was 205 mg. per 100 g. fresh liver weight. Animals receiving 10 per cent supplements of linoleic acid for eight days had liver free cholesterol values of 260 mg. per 100 g. fresh liver weight. Previous reports by J. Avigan and D. Steinberg (*Proc. Soc. Exp. Biol. Med.* **97,** *814 (1958)*) indicated that corn oil feeding increased the esterified cholesterol of rat livers but that no significant changes occurred in liver cholesterol of rats fed coconut oil.

Fecal excretion of Lieberman-Burchard chromogens increased by 47 per cent in linoleic acid-fed rats as compared to those receiving the stock ration. Similarly, the excretion of digitonin precipitable 3-beta-hydroxy sterols increased 87 per cent and bile acids increased 100 per cent. Addition of coconut oil resulted in a slight decrease in Lieberman-Burchard chromogens, but there was a 20 per cent increase in excretion of bile acids.

This type of approach may help to explain some of the paradoxes resulting from linoleic acid feeding. It would appear that the synthesis of cholesterol by the liver is considerably increased under these circumstances and that cholesterol catabolism is also greatly accelerated.

It is difficult to compare the effects of linoleic acid feeding on the anabolic and catabolic aspects of cholesterol metabolism

in this study. But it does seem that the saturated fatty acids in coconut oil produce an opposite effect on cholesterol synthesis and a similar but less marked effect on cholesterol catabolism. Efforts to measure cholesterol synthesis and cholesterol breakdown simultaneously and in comparable units are to be encouraged.

AN ENDOCRINE EFFECT OF NUTRITIONAL DEFICIENCIES—DEPRESSION OF HEPATIC INSULINASE

Rats deficient in pantothenic acid or protein or riboflavin have less hepatic insulinase than normally. Deficiency of thiamine is without effect.

Under conditions of semi-starvation or malnutrition, mature men have been observed to develop some degree of feminization. This clinical observation gains significance when one considers certain mechanisms of endocrine control. In rats, estrogenic substances are normally inactivated by the liver, usually by esterification. Whether this same mechanism applies to man is debatable. Nevertheless, when men are given a diet which is inadequate or deficient for a prolonged period of time, evidences compatible with greater estrogenic activity become manifest in loss of libido, gynecomastia and decreased growth of beard. Contrary to expectation, however, vascular spiders, often seen in conditions of estrogenic excess, were reported by W. B. Bean to be fewer per person in patients with nutritional deficiencies than in patients with chronic disease of the liver (*Vascular Spiders and Related Lesions of the Skin, p. 83. Charles C. Thomas, Springfield, Illinois (1958)*). Hepatic integrity, both nutritional and anatomic, seems to be necessary to maintain a normal rate of estrogenic destruction.

Other hormones are known to be inactivated by the liver. Hepatic destruction of testosterone is a normal function which is decreased by half in rats deprived of both niacin and tryptophan (M. J. Bryson, L. T. Samuels and H. C. Goldthorpe (*Endocrinology* **47,** *89 (1950)*). Furthermore, the liver of normal animals serves as the chief inactivator of antidiuretic hormones (W. J. Eversole, J. H. Birnie and R. Gaunt, *Ibid.* **45,** *378 (1949)*). This may be one of the factors responsible for the formation of ascites by patients with Laennec's cirrhosis (E. P. Ralli *et al., J. Clin. Invest.* **24,** *316 (1945)*). Other studies of rats deficient in protein or certain vitamins have shown that they respond poorly to the diuretic stimulus of water loading.

A few years ago, I. A. Mirsky, G. Perisutti, and R. Jinks (*Endocrinology* **56,** *484 (1955)*) demonstrated that the liver of normal animals contains a substance which will inactivate insulin. By inference, one might suspect that the apparent oversensitivity to insulin encountered in certain forms of hepatic disease could be due in part to impairment of insulinase activity. More likely however, is the fact that an injured liver cannot perform gluconeogenesis or store glycogen properly.

In a recent report, D. Diengott, S. Halevy and K. Guggenheim (*Endocrinology* **65,** *602 (1959)*) described the effects of deficiencies of certain nutrients upon the hepatic insulinase of rats. Young white male rats were fed a normal diet or diets deficient in protein, thiamine, riboflavin or pantothenic acid. The group deficient in pantothenic acid was given 15 mg. of the calcium salt of omega-methylpantothenic acid per kg. of food, and the group deficient in riboflavin was given 50 mg. of galactoflavin per kg. of food. Protein was fed as casein in amounts of 0, 6, 12 or 18 per cent of the dietary weight. Insulinase activity was determined by the method described by Mirsky

(*loc. cit.*). This activity was expressed as the number of units of insulin destroyed by a gram of liver homogenate in 30 minutes at 37°C., and as units destroyed per gram of hepatic nitrogen. The authors wisely employed two groups of control animals; one group was pair-fed and the other was fed ad libitum.

The time it took for the experimental animals to develop evidences of deficiencies was as follows: two weeks for protein, four to five weeks for thiamine, six to eight weeks for pantothenic acid and eight to ten weeks for riboflavin. The animals were then sacrificed.

Pair-fed animals consistently had less hepatic insulinase than did the animals allowed to eat more, although the difference was slight. This also demonstrated another characteristic of hepatic insulinase. As the rats grew older and heavier their hepatic insulinase increased in concentration (both in units of insulin destroyed per gram of liver and in units destroyed per gram of hepatic nitrogen). Increasing increments of protein in the diet (from 0 to 18 per cent) resulted in significant progressive increments of hepatic insulinase.

Rats deficient in thiamine did not have impairment of hepatic insulinase function. However, those deficient in pantothenic acid or protein or riboflavin were found to have significant impairment of this enzymatic function. Riboflavin deficiency exerted the greatest effect.

The concept that hormonal actions can be potentiated by dietary deprivation is a useful one. It seems evident that several enzyme systems are involved, since a deficiency of thiamine had no effect upon insulinase but did impair inactivation of antidiuretic hormones (Guggenheim, *Metabolism* **3,** *44* (*1954*)). Moreover, the findings of Diengott and co-workers may have significant bearing upon clinical as well as experimental problems. For example, the diabetic patient who has been labeled "brittle" because of his perverse habit of varying from a state of hypoglycemia to one of ketosis, can often benefit from an increase in his dietary protein. While most clinicians have considered that improvement resulted from a more gradual availability of carbohydrates, it is possible that more protein may provide a more constant supply of hepatic insulinase.

Furthermore, reports have appeared of abnormally low levels of blood sugar in rats deficient in riboflavin (B. R. Forker and A. F. Morgan, *J. Biol. Chem.* **209,** *303* (*1954*)) or pantothenic acid (L. S. Hurley and J. B. Mackenzie, *J. Nutrition* **54,** *403* (*1954*)).

In human pantothenic acid deficiency, a combination of findings appeared to indicate impairment of adrenal cortical function (W. B. Bean, R. E. Hodges, and K. Daum, *J. Clin. Invest.* **34,** *1073* (*1955*)). However, in later studies, this group found evidence that the adrenal cortex was functionally intact, and yet an exaggerated sensitivity to insulin was consistently observed (*Ibid.* **37,** *1642* (*1958*); **38,** *1421* (*1959*)). The suggestion that pantothenic acid deficiency resulted in impairment of hepatic insulinase activity seems plausible.

Although deficiency of riboflavin resulted in the most marked degree of inhibition of insulinase, it took the longest period of time to develop. For this reason, a study of the several types of deficiencies for comparable periods of time would be desirable.

The biochemical actions of pantothenic acid, which functions as coenzyme A in the varied process of acetylation, and of riboflavin, which has multiple enzymatic functions, seem to have little if anything in common. Neither is it clear whether a deficiency of protein induces the same or different changes in the obscure process of manufacture of insulinase. The article by Diengott, Halevy and Guggenheim makes a significant contribution to the study of nutritional requirements and carbohydrate metabolism.

NOTES

Letter to the Editor

Dear Sir:

The results of our investigation (*J. Nutrition* **67,** *333* (*1959*)) that were cited in a recent review (*Nutrition Reviews* **17,***340* (*1959*)) were not accurately interpreted. All of the evidence presented in our study would support the view that vitamin E is functioning solely as an antioxidant. Nevertheless the article was summarized in the review as "indicating the action of vitamin E is not simply as an antioxidant." A greater effectiveness of alpha-tocopherol on a molar basis when administered orally we believe is a reflection of greater retention of this antioxidant in fatty tissue compared to synthetic materials such as 1,2-dihydro-6-ethoxy 2,2,4-trimethylquinoline (ethoxyquin) and N,N′ diphenyl-para-phenylenediamine (DPPD).

There has been a large number of reports in the last two years demonstrating that these particular antioxidants or methylene blue have been able to replace vitamin E in the nutrition of the chicken and the rat. When negative results are observed with antioxidants, these can be attributed to (1) using an antioxidant which does not have this property such as 2,6-ditertiarybutyl-4-methoxyphenol (BHA), 2,6-ditertiarybutyl-4-methylphenol (BHT), or propyl gallate; (2) using too low a level of antioxidant; or (3) using a level of methylene blue or N,N′ diphenyl-para-phenylenediamine (DPPD) which results in toxicity and thereby prevents normal function of the animal.

There was another minor inaccuracy which we would like to correct. The reviewer consistently considered calcium DL-2-hydroxy-4-methyl thiobutyrate as an antioxidant. This material is the hydroxy analogue of methionine. Previous work (*J. Nutrition* **60,** *87* (*1956*)) demonstrated that appearance of muscular dystrophy of nutritional origin in the chick is dependent on a sulfur amino acid deficiency. We do not believe, nor did we state in our paper, that this material functions other than to remedy the sulfur amino acid deficiency of the diets employed.

In our more recent work, we have found that dietary linoleic acid is a causative agent of encephalomalacia (*Poultry Sci.* **38,** *1224* (*1959*)). This knowledge coupled with the known role of selenium in preventing exudative diathesis has allowed us, by the following dietary treatments, to separate and produce at will any of the classical symptoms of "vitamin E" deficiency: (1) muscular dystrophy, low sulfur amino acids, low antioxidants; (2) exudative diathesis, low selenium, low antioxidants, high linolenic acid; (3) encephalomalacia, high linoleic acid, low antioxidants. In every case a suitable level of an antioxidant such as ethoxyquin will completely prevent the "vitamin E" deficiency symptoms.

In summary, we believe that all evidence to date indicates that vitamin E should be considered as a naturally-occurring biologically active antioxidant. Therefore, until data are available to the contrary, no specific or unique function can be assigned to this vitamin.

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Effects of Excesses of Thiamine and Pyridoxine

Almost without exception, excessive doses of water soluble vitamins, even when given for prolonged periods of time, have been without detectable deleterious effect. However, M. B. Richards (*Brit. Med. J.* **1,** *433* (*1945*)) reported that excessive amounts of thiamine given to female rats fed a white

flour and casein diet resulted in convulsions and a high rate of mortality in their offspring. This could be prevented, however, by giving pyridoxine to the lactating mothers.

Moreover, A.D. Hunt, Jr., *et al.* (*Pediatrics* **13,** *140 (1954)*) cared for an infant suffering from convulsions, whose mother had taken excessive amounts of pyridoxine during pregnancy. They relieved these convulsions by administration of pyridoxine, leading them to postulate that excessive intake of pyridoxine during pregnancy may increase the infant's requirement for pyridoxine after birth.

In a study of this problem, A. B. Morrison and H. P. Sarett (*J. Nutrition* **69,** *111 (1959)*), gave excessive amounts of thiamine or pyridoxine or both (at 50 times the basal levels) to female rats. They recorded their rate of growth, reproduction performance and vitamin stores, and estimated these effects in the offspring.

The rates of growth and efficiency of food utilization in the mothers were unaffected by excess doses of the vitamins, and the average size, survival and growth of the offspring were unchanged as well.

However when both thiamine or pyridoxine were given together, the number of offspring increased slightly. There was no evidence of pyridoxine storage in the mothers, although there was considerable hepatic storage of thiamine.

When offspring of mothers who had received excess pyridoxine were fed a diet deficient in pyridoxine, they grew better than the group whose mothers had eaten a basal diet.

Determinations were made of hepatic thiamine and pyridoxine in the young rats at weaning and after two and five weeks of a pyridoxine-deficient diet. At time of weaning, the livers contained more thiamine or pyridoxine than the respective controls, although the quantity of pyridoxine was less in the young than it was in the mother.

Thus these data did not support the hypothesis of Hunt that excessive feeding of pyridoxine to mothers can increase the requirement of the offspring. However, it is conceivable that humans metabolize this vitamin in a different manner than do rats.

Recent Books

Nutrients in Frozen Foods; Frozen Foods for Family Meals. Prepared by Dr. Miriam E. Lowenberg and Dr. Eva D. Wilson, College of Home Economics, Pennsylvania State University, University Park, Pennsylvania. Pp. 9.

Variation in Dental Caries Experience Among Children of Five Western States. Cooperative Nutritional Status Studies in the Western Region III. By Gertrude Tank, Nettie C. Esselbaugh, Kathleen P. Warnick and Clara A. Storvick, Agricultural Experiment Station, Oregon State College, Corvallis, Oregon. Pp. 35.

Chemistry of Plant Gums and Mucilages and Some Related Polysaccharides. By F. Smith and R. Montgomery. Published by Reinhold Publishing Corp., 430 Park Avenue, New York; Chapman & Hall, Ltd., London. Pp. 627.

Manioc in Africa. By William O. Jones. A publication of the Food Research Institute, Stanford University Press, Stanford, California. Pp. 301. Price $6.75.

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